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Dual Action of the Neuropeptide Galanin on the Cytoplasmic Free Calcium Concentration in RIN m5F Cells

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Summary. The neuropeptide, galanin, potently inhibits insulin secretion and is thought to be an adrenergic neurotransmitter in the pancreas. In this study, the effects of galanin and the galanin receptor antagonist, galantide, on the cytoplasmic free Ca^{2+} $[Ca^{2+}]_i$, were investigated in FURA 2-AM-loaded cells of the rat insulinoma cell line, RIN m5F, in cell suspensions in a cuvette. It was found that galanin (100 nmol/l) after a prior addition of D-glyceraldehyde (15 mmol/l), both at 3.3 and 8.3 mmol/l glucose, induced a dual, biphasic, action on the $[Ca^{2+}]_i$: a rapid and transient peak was followed by a reduction below the prestimulatory levels. The rapid peak was similar to that induced by the cholinergic agonist, carbachol (100 μmol/l). A prior addition of the galanin receptor antagonist, galantide (500 nmol/l), abolished the changes in $[Ca^{2+}]_i$ after galanin. However, galantide by itself induced the same biphasic changes in $[Ca^{2+}]_i$ as those induced by galanin. Hence, the study demonstrates a)that galanin induces a dual response in $[Ca^{2+}]_i$ in insulin-producing RIN m5F cells with a rapid peak preceeding a reduction below prestimulatory levels, and b)that the galanin receptor antagonist, galantide, is a partial galanin agonist. It is proposed that the changes in $[Ca^{2+}]_i$ induced by galanin are much more complex than previously thought and, therefore, that galanin does not inhibit insulin secretion by simply reducing the $[Ca^{2+}]_i$.

As recently reviewed, the neuropeptide galanin is located in adrenergic nerve terminals in the pancreatic islets and potently inhibits glucose-stimulated insulin secretion under a variety of experimental conditions (1). The neuropeptide has therefore been hypothesized to be an islet neurotransmitter involved in the sympathetic (adrenergic) regulation of insulin secretion (1-3).

The cytoplasmic free concentration of calcium, ($[Ca^{2+}]_i$), is known to be an important determinant for glucose-stimulated insulin secretion (4) and the mechanism underlying the potent inhibitory action of galanin on insulin secretion has been thought to involve reduced $[Ca^{2+}]_i$. Thus, the neuropeptide has been demonstrated to inhibit the glucose-stimulated increase in $[Ca^{2+}]_i$ in quin 2-loaded isolated islets cells from the obese hyperglycemic ob/ob mouse (5,6) as well as the increase in $[Ca^{2+}]_i$ in fura 2-loaded isolated rat islet cells stimulated by the octapeptide of cholecystokinin (CCK-8) (7). Galanin has also been demontrated to inhibit the phorbol ester-stimulated increase in $[Ca^{2+}]_i$ in the rat insulinoma cell line, RIN m5F cells, concomitantly with inhibiting insulin secretion (8).

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Galanin has been thought to reduce $[Ca^{2+}]_i$ by hyperpolarization-induced closure of voltage-sensitive Ca^{2+} channels. The chain of events is thought to include opening of membraneous K^+ channels, which leads to hyperpolarization which in turn closes the voltage-sensitive Ca^{2+}-channels and reduces the Ca^{2+} entry (for review see 1). In support of this assumption, galanin has been demonstrated to induce hyperpolarization, and the neuropeptide has been shown to be unable to reduce the increase in $[Ca^{2+}]_i$ that follows depolarization by KCl (5). Furthermore, patch clamp studies have shown that galanin directly opens membraneous K^+-channels (9), and in $^{86}Rb^+$-preloaded normal rat islets, galanin stimulates to a subsequent $^{86}Rb^+$-efflux, a sign that reflects opening of K^+ channels (10). In RIN m5F cells, galanin seems also, however, to inhibit the Ca^{2+} entry by directly blocking the activity of the membraneous Ca^{2+} channels (11), indicating the existence of more than one mechanism of the neuropeptide on ion channel activity.

Recently, a new tool for the study of galanin mechanisms was invented since a series of chimeric peptides with galanin receptor antagonistic properties was synthesized (12). One of these peptides is galantide, which was demonstrated to displace galanin from the receptors in RIN m5F cells and to counteract the inhibitory action of galanin on glucose-stimulated insulin secretion in normal rats islets (13) as well as to be a reliable galanin receptor antagonist in the rat hippocampus (14).

The present study was undertaken to examine whether galantide affects also the galanin-induced reduction in $[Ca^{2+}]_i$ in RIN m5F cells. Beside this, the study also examined in more detail the action of galanin on $[Ca^{2+}]_i$.

MATERIALS and METHODS

Materials. Cells of the rat insulinoma cell line, RIN m5F, were cultured in RPMI 1640 medium supplemented with fetal calf serum (10%), 2.06 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml amphotericin B (GIBCO, Life Technologies Ltd., Paisley, Scotland) at 37°C in air- 5% CO_2 athmosphere. Cells at the passage number 90-130 were used.

Fura-2-AM studies. Cultured RIN m5F cells were dispersed into single cells by trypsinization. The single cells were suspended at $0.5x10^6$ cells/ml in RPMI 1640 medium supplemented with 10% fetal calf serum for 60 min. The cell suspension was then loaded for 45 min in the RPMI 1640 medium together with 1 μmol/l FURA 2-AM (Sigma Chemical Co., St. Louis, Mo, USA). Thereafter, the cells were rinsed twice and gently shaken for 20 min in the experimental medium, consisting of 125 mmol/l NaCl, 1.28 mmol/l $CaCl_2$, 5.9 mmol/l KCl, 1.2 mmol/l $MgCl_2$ and 25 mmol/l Hepes. This procedure allows equilibration of the $[Ca^{2+}]_i$.

The cells were then washed and resuspended in the experimental medium ($0.6x10^6$ cells/ml). Two ml of the cell suspension were then transferred to a cuvette in a Perkin-Elmer LS-50 spectrophotofluorometer for measurement of $[Ca^{2+}]_i$. The cell suspension was constantly stirred with a stirrbar mounted at the side of the cuvette. The excitation wavelengths were 340 and 380 nm and the emmision wavelength was 490 nm. The ratio between 340 and 380 nm was calculated using softwares designed by Perkin-Elmer ("Intracellular Biochemistry"). Fluorescence maximum was obtained by adding 0.03% triton (Triton X-100) and fluorescence minimum by adding 8.5 mmol/l EGTA (both British Drug Houses Ltd., Poole, England) at the end of each experiment. $[Ca^{2+}]_i$ was then calculated according to the formula $[Ca^{2+}]_i = K_D(R-R_{min}/R_{max}-R)(S_{f2})/(S_{b2})$, as initially described by Grynkiewics et al. (15). K_D was assumed to be 224 nmol/l.

Synthetic rat galanin was obtained from Peninsula Laboratories (Belmonte, Ca., U.S.A.), D-glyceraldehyde and carbachol were from British Drug Houses Ltd., Poole, England), and synthetic galantide was a kind gift from Professor Tamas Bartfai and Dr Ulo Langel, Department of Biochemistry, Stockholm University, Stockholm.

RESULTS

In the presence of 3.3 mmol/l glucose (Fig. 1) as well as in the presence of 8.3 mmol/l glucose (Fig. 2), glyceraldehyde (15 mmol/l) induced a gradual increase in $[Ca^{2+}]_i$ when introduced into the cell suspension. Under both conditions, a subsequent introduction of galanin (100 nmol/l) induced a short-lived and prompt peak in $[Ca^{2+}]_i$ followed by a reduction of $[Ca^{2+}]_i$ to values below the prestimulatory level. A later introduction of carbachol (100 μmol/l) was followed by a marked, though transient, increase in $[Ca^{2+}]_i$ without any reduction below prestimulatory levels after the peak. Fig. 3 shows that an initial introduction of galanin (100 nmol/l) at 8.3 mmol/l glucose was followed by the same dual pattern of change in $[Ca^{2+}]_i$ as when galanin was introduced after glyceraldehyde (cf. Fig. 1). Furthermore, introduction of galanin before glyceraldehyde did not affect the response to glyceraldehyde (Fig. 3).

When introducing the galanin receptor antagonist, galantide (500 nmol/ l), at 3.3 mmol/l glucose after introduction of glyceraldehyde (Fig. 4), the $[Ca^{2+}]_i$ was transiently increased followed by a post-peak reduction below baseline level, i.e., the same pattern of change was seen after galantide as after galanin (cf. Figs. 1 and 2). However, a subsequent introduction of galanin did not affect $[Ca^{2+}]_i$, i.e., the antagonist prevented the changes in $[Ca^{2+}]_i$ induced by galanin (Fig. 4).

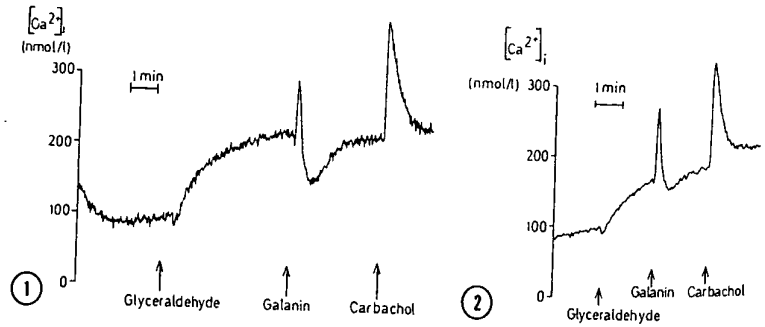

Fig. 1. Effects of D-glyceraldehyde (15 mmol/l), synthetic rat galanin (100 nmol/l) and the cholinergic agonist carbachol (100 μmol/l) on $[Ca^{2+}]_i$ in FURA 2-AM-loaded RIN m5F cells in a medium containing 3.3 mmol/l glucose.

Fig. 2. Effects of D-glyceraldehyde (15 mmol/l), synthetic rat galanin (100 nmol/l) and the cholinergic agonist carbachol (100 μmol/l) on $[Ca^{2+}]_i$ in FURA 2-AM-loaded RIN m5F cells in a medium containing 8.3 mmol/l glucose.

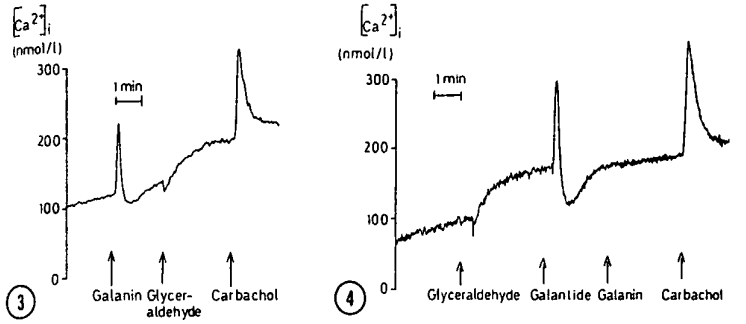

Fig. 3. Effects of synthetic rat galanin (100 nmol/l), D-glyceraldehyde (15 mmol/l) and the cholinergic agonist carbachol (100 μmol/l) on $[Ca^{2+}]_i$ in FURA 2-AM-loaded RIN m5F cells in a medium containing 8.3 mmol/l glucose.

Fig. 4. Effects of D-glyceraldehyde (15 mmol/l), the synthetic galanin receptor antagonist, galantide (500 nmol/l), synthetic rat galanin (100 nmol/l) and the cholinergic agonist carbachol (100 μmol/l) on $[Ca^{2+}]_i$ in FURA 2-AM-loaded RIN m5F cells in a medium containing 3.3 mmol/l glucose.

DISCUSSION

It was previously thought that galanin simply reduces the $[Ca^{2+}]_i$ in insulin producing cells, as inferred from results in studies on quin 2-loaded ß-cells from the hyperglycemic mouse (5,6), on fura 2-loaded RIN m5F cells (8), and on normal $^{45}Ca^{2+}$-preloaded rat islets (10). However, the present study demonstrates that the effects of galanin on $[Ca^{2+}]_i$ in insulin producing cells are more complex than previously thought. Thus, we demonstrate that the neuropeptide induces a dual action on the RIN m5F cell $[Ca^{2+}]_i$ with a rapid peak preceeding the inhibition. The second phase (inhibition) confirms the previous studies, and particularly the previous study on RIN m5F cells, which showed that at 120 seconds after introduction of galanin to a RIN m5F cell suspension, the $[Ca^{2+}]_i$ had been lowered (8). However, that study did not report any data on the initial action after introduction of galanin, which is the time point where we demonstrate the rapid peak.

The reduction of $[Ca^{2+}]_i$ after introduction of galanin has been explained by the hyperpolarizing effect of the peptide through opening of membraneous K^+-channels (1), although a direct blocking action of membraneous Ca^{2+} channels might contribute as well, at least in RIN m5F cells (11). In contrast, from the knowledge of actions of galanin in insulin producing cells, the stimulatory peak in $[Ca^{2+}]_i$ induced by the peptide is more difficult to explain. The peak shows resemblences to that induced by carbachol (see Figs.), i.e., it is very rapid and transient. Cholinergic agonists are known to stimulate phosphoinositide hydrolysis in insulin producing cells (16,17), which induces the formation of inositol-1,4,5-trisphosphate, which in turn stimulates the release of Ca^{2+} from intracellular storage sites causing the $[Ca^{2+}]_i$ to increase rapidly (4). A similar mechanism has been proposed for the rapid peak in $[Ca^{2+}]_i$

induced by CCK-8 in islet cells (18). Hence, though other actions are as well possible, our study therefore might indicate that also galanin induces phosphoinositide hydrolysis in RIN m5F cells. In other systems, the action of the neuropeptide in this respect seems different depending on the system under study. For example, in cardiac tissue of the mudpuppy, galanin stimulates phospatidylinositol turnover (19), whereas in slices of monkey hippocampus, acetylcholine-stimulated phosphoinositide turnover is inhibited by the peptide (20).

The transient peak in $[Ca^{2+}]_i$ induced by galanin was previously not demonstrated in ß-cells loaded with quin-2 (5,6) or fura-2 (7), although in the latter study (7), galanin was added only during non-stimulatory conditions. Concerning the quin-2-loaded cells, it might be anticipated that the characteristically slow reactiveness of quin-2 (21) might have masked such a rapid and short-lived peak as that induced by galanin. Hence, by the use of more optimal conditions (fura 2-loading, adding galanin under stimulatory conditions and determining the temporal action on $[Ca^{2+}]_i$), we have been able to demonstrate the dual action of galanin on $[Ca^{2+}]_i$ in insulin-producing cells. Hence, the suggestion that galanin inhibits insulin secretion simply by reducing the $[Ca^{2+}]_i$ seems to be an oversimplification. It should also be mentioned here that other mechanisms as well have been demonstrated by the neuropeptide in insulin producing cells. Thus, galanin inhibits cyclic AMP production in normal islets (10) and the adenylate cyclase activity in RIN m5F cells (22), and galanin also inhibits insulin secretion by a direct action on the exocytosis machinery (23). Hence, a multitude of actions seem to underlie the inhibitory effect of galanin on insulin secretion (1).

Galantide is a 20 amino acid which previously has been demonstrated to block galanin-induced inhibition of glucose-stimulated insulin secretion in isolated mouse islets and to displace iodinated galanin from membranes of RIN m5F cells (13). Galantide has also been shown to block neuronal effects of galanin in the hippocampus, locus coeruleus and spinal cord (14) and to prevent galanin-evoked acetylcholine release in the rat striatum (24). Galantide consists of galanin(1-13) coupled to substance P(5-11). It belongs to a series of chimeric peptides which are ligands to galanin and substance P receptors with putative actions as receptor antagonists (12,25). We show in this study that galantide prevents galanin-induced actions also on $[Ca^{2+}]_i$ in RIN m5F cells, thus confirming the galanin receptor antagonistic property of the peptide. However, we also show that galantide in itself induces changes in $[Ca^{2+}]_i$ that are equivalent to those induced by galanin. Hence, galantide seems in RIN m5F cells to be a partial galanin agonist which also functions as an antagonist, presumably due to a higher affinity than galanin for the galanin receptors.

In conclusion, we show in this report that galanin induces a biphasic response in $[Ca^{2+}]_i$ in RIN m5F cells and that the galanin receptor antagonist, galantide, is a partial galanin receptor agonist in these cells.

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